Virilism and Ectopic Expression of HSD17B5 in Mature Cystic Teratoma

Yohei Kawaguchi,[1] Hiroko Mizuno,[1] Mai Horikawa,[2] Mayuko Kano,[1] Kengo Yamada,[1] Fumiko Yamakawa,[1] Takashi Maekawa,[3] Yuto Yamazaki,[3] Keely M. McNamara,[3] Hironobu Sasano[3] and Masayuki Hayashi[1]

[1]Department of Endocrinology and Diabetes, Japan Community Health care Organization Chukyo Hospital, Nagoya, Aichi, Japan
[2]Department of Obstetrics and Gynecology, Japan Community Health care Organization Chukyo Hospital, Nagoya, Aichi, Japan
[3]Department of Pathology, Tohoku University Graduate School of Medicine, Sendai, Miyagi, Japan

Mature cystic teratoma (MCT) is rarely involved in the overproduction of steroid hormones in contrast to sex cord stromal tumors. A 31-year-old woman visited our hospital with hirsutism, hoarseness, and hair loss from the scalp. Serum testosterone and free-testosterone levels were 7.3 ng/ml and 2.3 pg/ml, respectively, which were markedly in excess of the age adjusted female standard levels. Basal blood levels of steroid hormones and serum levels of 17-hydroxyprogesterone at 1 h after intravenous injection of adrenocorticotropic hormone demonstrated that 21-hydroxylase deficiency was not the underlying cause of her virilization. A subsequent chromosomal test with G-banding revealed a karyotype of 46XX. Magnetic resonance imaging revealed a mass in the left ovary, which was subsequently diagnosed as MCT. Detailed pathological analysis of the tumor indicated that it was comprised of skin components, sweat glands, with hair and fat texture, glandular epithelium and fibrous connective tissue, consistent with the characteristic composition of MCT. Immunohistochemical analysis demonstrated marked immunoreactivity of 17beta-hydroxysteroid dehydrogenase (HSD17B5), an enzyme that can convert androstenedione to testosterone. Following surgical removal of the tumor, testosterone and free testosterone levels were markedly decreased (0.3 ng/ml and 0.4 pg/ml, respectively) and other symptoms abated. In conclusion, this is the first report of an ovarian MCT associated with clinical virilization caused by the ectopic production of testosterone possibly because of an overexpression of intratumoral HSD17B5.

Introduction

Mature cystic teratoma (MCT), a common disease in reproductive age women, is a highly differentiated tumor with two or three intermingled germ layer components (Comerci et al. 1994). Such tumors are commonly asymptomatic but occasionally are accompanied by severe pain from reactive peritonitis caused by either torsion associated with the MCT or irritation of the cyst wall by sebaceous materials secreted from the MCT (Hoffman et al. 2009). MCT harbors many different components including hair, skin, bone, fat and endocrine cells (e.g. struma ovarii) but it is clinically apparent that the ectopic production of hormones, especially steroid hormones, is rare (Tomlinson et al. 1996). Here, we report a case of ovarian MCT associated with clinically evident virilization caused by increased

testosterone production via the ectopic expression of 17beta-hydroxysteroid dehydrogenase (HSD17B5).

Case Presentation

A 31-year-old nulligravid woman was referred to our hospital from a dermatology unit because of hypertrichosis and virilism. Her age at menarche was 11 years but her menstrual cycle had been irregular. She self noted hirsutism after her first menarche, and showed gradually progressive hair loss after 30 years of age. She had no significant family history of endocrine diseases. On her initial visit, her weight was 60 kg and her height was 148 cm, with a body mass index of 29.7 kg/m^2, classifying her as obese. Observationally she had hair loss, a beard, hirsutism, and a low voice. Her underarm hair, pubic hair, and external genital were within normal ranges. Blood examination indi-

cated that basal levels of testosterone (7.3 ng/ml) and free-testosterone (2.3 ng/ml) were elevated, while those of dehydroepiandrosterone sulfate (200 μg/dL), luteinizing hormone (LH; 8.03 mIU/ml), follicle-stimulating hormone (FSH; 3.09 mIU/ml) and other pituitary and adrenal hormones were within the normal age-matched ranges (Table 1). Abdominal magnetic resonance imaging (MRI) revealed a left ovarian polycystic mass measuring 44 × 33 mm with calcification and fat, many small cysts in both ovaries (Fig. 1A), and a left adrenal mass measuring 7.5 × 9.0 mm (Fig. 1B). A 1 mg dexamethasone suppression test following overnight fast was also within normal limits, suggesting that the adrenal tumor was non-functioning. Basal blood levels of steroid hormones were in the normal range, and changes of serum levels of 17-hydroxyprogesterone at 1 h

after intravenous injection of adrenocorticotropic hormone were 2.1 ng/mL (less than 10 ng/mL), suggesting the absence of 21-hydroxylase deficiency (Armengaud et al. 2009) (Table 2). A luteinizing hormone-releasing hormone loading test revealed a delayed response, which is not consistent with that in polycystic ovary syndrome (PCOS) (Table 1). Chromosomal test with G-banding revealed a normal female karyotype of 46XX. After the above diagnostic test, the ovarian tumor was determined to be the principle contributing factor to the elevated testosterone level, and cystectomy of the ovarian tumor was subsequently performed without any complications. After surgery, the basal levels of testosterone and free-testosterone were significantly decreased (0.3 ng/ml and 0.4 pg/ml, respectively), and the symptoms of hirsutism, low voice

Table 1. Laboratory data on admission.

Biochemistry

Blood urea nitrogen	11 mg/dl	Triglyceride	83 mg/dl
Creatinine	0.45 mg/dl	High-density lipoprotein	65 mg/dl
Uric acid	5.4 mg/dl	Low-density lipoprotein	132 mg/dl
Sodium	137 mEq/l	Blood sugar	85 mg/dl
Potassium	4.6 mEq/l	HbA1c	5.7 %
Chloride	104 mEq/l		

Endocrinology

Growth hormone	0.555	ng/ml	Plasma renin activity	1.6	ng/ml/hr
Insulin-like growth factor 1	225	ng/ml	Aldosterone	124	pg/ml
Adrenocorticotropic hormone	5.8	pg/ml	Adrenaline	0.03	ng/ml
Cortisol	5.3	μg/ml	Noradrenaline	0.11	ng/ml
Luteinizing hormone	8.03	mIU/ml	Dopamine	0.02	ng/ml
Follicle-stimulating hormone	3.90	mIU/ml	Vanilylmadelic acid	7.1	ng/ml
Dehydroepiandrosterone sulfate	200	μg/dl	Homovanillic acid	9.1	ng/ml
Testosterone	7.30	ng/ml	Thyroid stimulating hormone	1.010	μIU/ml
Free testosterone	2.30	pg/ml	Free triiodothyronine	3.3	pg/ml
Estradiol	49	pg/ml	Free thyroxine	1.2	ng/dl
Progesterone	0.3	ng/ml	Prolactin	10.13	ng/ml
Human chorionic gonadotropin	<1.0	mIU/ml			
Pregnenolone	0.14	ng/ml	(0.2-1.5)		
Deoxycorticosterone	0.09	ng/ml	(0.03-3.00)		
Corticosterone	0.98	ng/ml	(0.21-8.48)		
17-hydroxypregnenolone	1.03	ng/ml	(0.2-4.5)		
17-hydroxyprogesterone	0.7	ng/ml	(0.2-4.5)		
11-deoxycortisol	<0.04	ng/ml	(0.11-0.60)		
Androstenedione	3.2	ng/ml	(0.9-3.5)		

The underlined numbers are deviated from normal ranges. Serum testosterone and free testosterone levels were much higher than the female standard levels. Basal blood levels of steroid hormones were in the normal range.
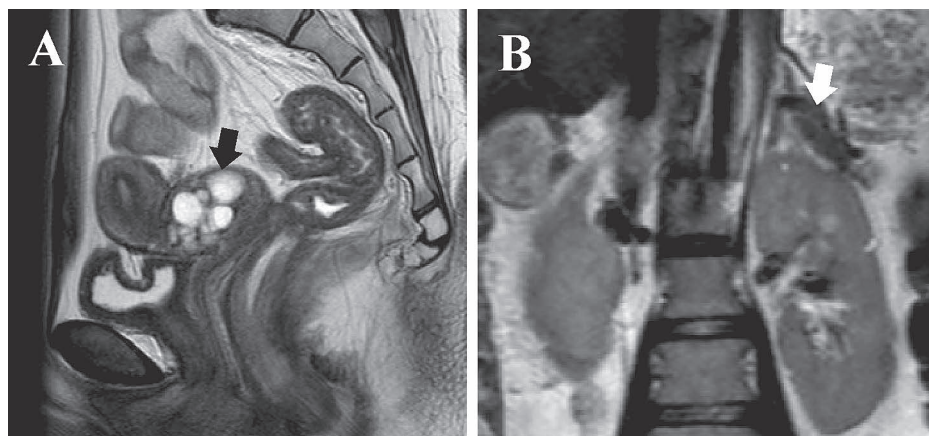
Fig. 1. Magnetic resonance imaging of the abdomen. The arrow indicates the left ovarian polycystic mass measuring 44×33mm with calcification and fat (A). The arrow indicates the left adrenal mass measuring 7.5×9.0 mm (B).

Table 2. Endocrinological loading tests.

Adrenocorticotropic hormone loading test

Time (min)	0'	30'	60'
17-hydroxyprogesterone (ng/mL)	0.9	2.3	3.0

Luteinizing hormone-releasing hormone loading test

Time (min)	0'	30'	60'	90'	120'
Luteinizing hormone (mIU/ml)	9.46	39.73	42.34	45.09	27.84
Follicle-stimulating hormone (mIU/ml)	4.45	6.58	7.68	9.06	9.79

The serum levels of 17- hydroxyprogesterone at 1 h after intravenous injection of adrenocorticotropic hormone suggested the absence of 21-hydroxylase deficiency. Luteinizing hormone-releasing hormone loading test revealed a delayed response, which is not consistent with that in polycystic ovary syndrome.

and irregular menstruation gradually improved during several months. Detailed pathological and immunohistochemical analysis of the resected tumor demonstrated that the lesion was comprised of various dermal components including sweat glands, hair, adipocytes, glandular epithelium and fibrous connective tissue, but not distinctive for sebaceous glands. These findings are characteristic of MCTs, and the MCT in this study was largely composed of skin components (Fig. 2A, B). The presence of thyroid follicle immunohistchemically positive for thyroglobulin indicated thyroid tissue/struma ovarii (Fig. 2C, D); however, steroidogenic factor 1 (SF1), a transcription factor associated with steroidogenesis, and inhibin α (INHA), a glycoprotein hormone produced in gonadal tissue, were not detected (Fig. 2E, F). HSD17B5, an enzyme that converts androstenedione to testosterone, was immunolocalized some clusters of the cells in cyst wall and focally positive for epithelial cells (Nakamura et al. 2009). Chromogranin A was immunolocalized in the same clusters of the cells (Fig. 2G, H). We performed immunostaining manually, using a mouse monoclonal antibody against HSD17B5 (Sigma, A6229, clone; NP G6, A6, dilution 1:200) (Sasano et al. 1992). Antigen retrieval was performed by autoclav-

ing the tissues at 121°C for 5 min.

Consent for Publication

Written informed consent was obtained from the patient for publication of this case report and any accompanying images.

Discussion

This is the first reported case of ovarian MCT associated with clinical masculinization by elevated serum testosterone levels and immunolozalization of HSD17B5 in skin component of the cystic tumor. Resection of the tumor normalized serum testosterone levels, suggesting that the elevated serum testosterone level and masculinization were caused by the tumor. Previously reported mechanisms by which teratomas are known to cause masculinization include peripheral luteinization of stromal cells (Freedman and Scully 1970, Lopez-Beltran et al. 1997), differentiation to Leydig cells (Bulwa and Lewis 1971, Wu et al. 2008, Rotenberg et al. 2009), concurrent hyperthecosis (Aiman et al. 1977), Brenner tumor-containing MCT (Tsujimoto et al. 2011), and hilus cell hyperplasia (Rutgers and Scully 1986). None of these histological findings was detected during the
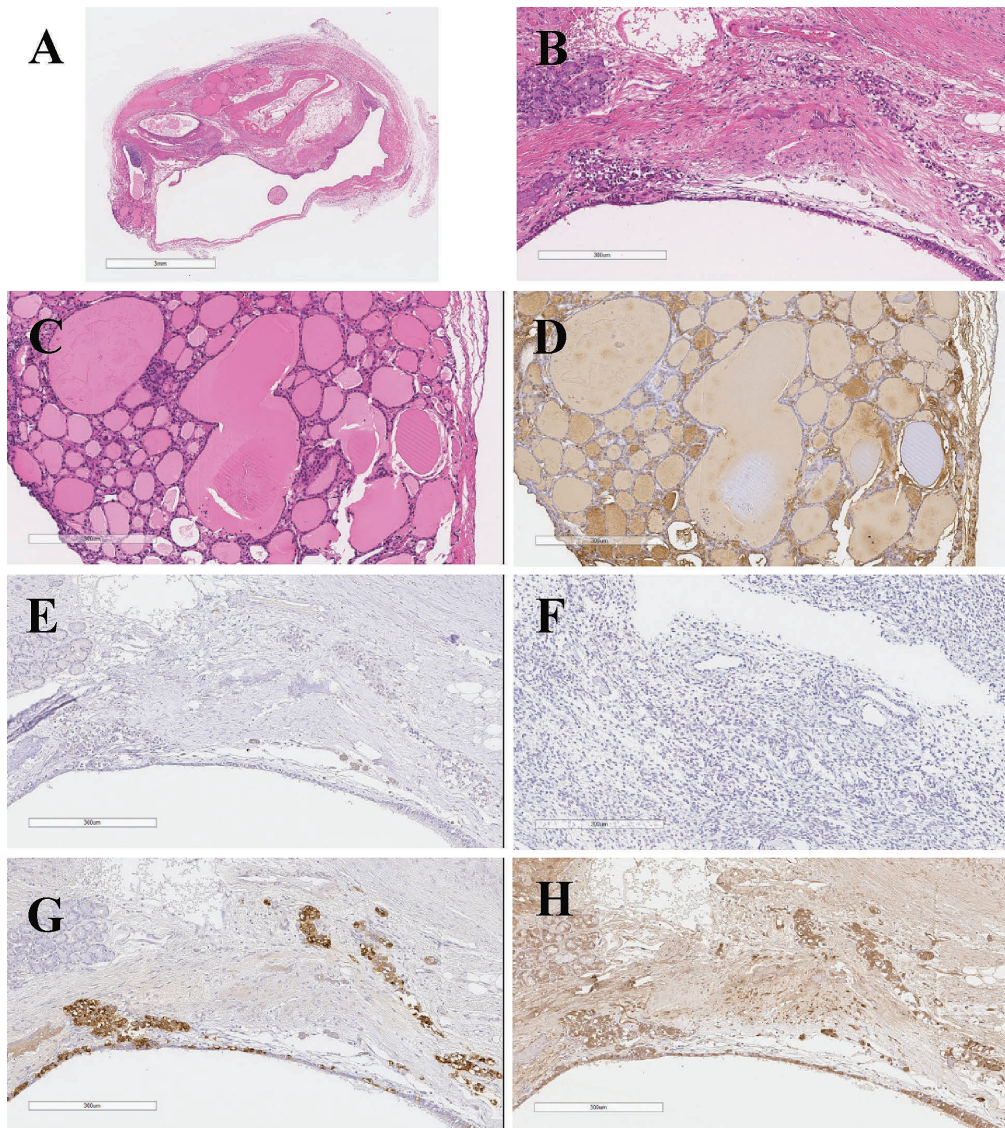
Fig. 2. Pathological analyses of the ovarian tumor. Hematoxylin and eosin staining of the resected tumor showed characteristic of mature cystic teratomas (MCTs) (A), and the MCT in this study was largely composed of skin components (B). The presence of thyroid follicle immunohistchemically positive for thyroglobulin indicated thyroid tissue/struma ovarii (C and D). Steroidogenic factor 1 (E) and inhibin α (F) were not detected in the specimens. Immuno-colocalization of Chromogranin A (G) and 17beta-hydroxysteroid dehydrogenase (HSD17B5, H), an enzyme that converts androstenedione to testosterone, was confirmed in some clusters of the cells in cyst wall and focally positive for epithelial cells. Original magnification: x loope (A), × 100 (B-H).

careful histopathological examination of the resected specimen. HSD17B5, which catalyzes the conversion of androstenedione to testosterone, was reported to be expressed in pathological ovarian tissues including PCOS or endometriosis and also detected in human normal ovary tissues (Ji et al. 2005). In this study, we selected HSD17B5 because of its pivotal roles in masculinization pathophysiology of these ovarian disorders as well as breast and prostatic malignancies, and the enzyme was immunolocalized in some clusters of the cells in cyst wall and focally positive for epithelial cells (Fig. 2G, H). A previous study reported that the epithelium surrounding the corpus luteum can produce dihydrotestosterone (Tsujimoto et al. 2011) and thus we cannot claim our MCT was the sole potential source of androgen production. However, the significant decrease in testosterone following surgery supports this abundant HSD17B5 immunoreactivity within the MCT as the major etiology underlying masculinization in this case.

PCOS is characterized by hyperandrogenism and is recognized as an important metabolic and reproductive disorder. Although serum LH and FSH levels stimulated by luteinizing hormone-releasing hormone and the clinical course after surgery are not consistent with PCOS in this case, indications of obesity in the present case, as well as the presence of hyperandrogenism, suggest that similar underlying mechanisms may be involved. Hyperinsulin-

emia reinforces testosterone action via insulin effects in the pituitary gland, liver, and ovary (Diamanti-Kandarakis and Papavassiliou 2006). Obesity or diabetes has been previously reported in cases of virilism with MCT (Aiman et al. 1977, Lopez-Beltran et al. 1997, Rotenberg et al. 2009). However, the long-term evaluation of diabetes and insulin resistance in this case could not be performed because of the patient's circumstances.

The production of hormones such as GH or HCG by MCTs has been reported, but is rare (Dawley et al. 2012, Ozkaya et al. 2015). The incidence of thyroid tissue in MCTs or struma ovarii have been reported to be 5-20% and 2.7% respectively, and clinically apparent production of thyroid hormone has been also reported in 5-6% of struma ovarii cases (Comerci et al. 1994). Thyroid tissues were detected in this tumor but serum thyroid hormone levels were normal.

Several previous pathological studies of sebaceous glands suggest a possible role for alterations of intracrine pathways in various human skin disorders, particularly acne (Thiboutot et al. 1998, Fritsch et al. 2001). In addition, steroidogenic enzymes including HSD17B5 have been reported in human skin (Azmahani et al. 2014). Therefore, it is reasonable that the skin components of this case of MCT might have been the cause of clinical symptoms of virilization in this patient.

In summary, we report a unique case of virilism accompanied by an ovarian cystic teratoma expressing elevated levels of HSD17B5. Normalization of elevated serum testosterone levels after its resection indicated that the overexpression of this enzyme could be, at least in part, the major cause of her virilization.

Conflict of Interest

The authors declare no conflict of interest.

References

Aiman, J., Nalick, R.H., Jacobs, A., Porter, J.C., Edman, C.D., Vellios, F. & MacDonald, P.C. (1977) The origin of androgen and estrogen in a virilized postmenopausal woman with bilateral benign cystic teratomas. *Obstet. Gynecol.*, **49**, 695-704.

Armengaud, J.B., Charkaluk, M.L., Trivin, C., Tardy, V., Breart, G., Brauner, R. & Chalumeau, M. (2009) Precocious pubarche: distinguishing late-onset congenital adrenal hyperplasia from premature adrenarche. *J. Clin. Endocrinol. Metab.*, **94**, 2835-2840.

Azmahani, A., Nakamura, Y., Felizola, S.J., Ozawa, Y., Ise, K., Inoue, T., McNamara, K.M., Doi, M., Okamura, H., Zouboulis, C.C., Aiba, S. & Sasano, H. (2014) Steroidogenic enzymes, their related transcription factors and nuclear receptors in human sebaceous glands under normal and pathological conditions. *J. Steroid Biochem. Mol. Biol.*, **144** Pt B, 268-279.

Bulwa, F.M. & Lewis, M.G. (1971) Ovarian teratoma containing arrhenoblastic tissue. *J. Obstet. Gynaecol. Br. Commonw.*, **78**, 759-761.

Comerci, J.T. Jr., Licciardi, F., Bergh, P.A., Gregori, C. & Breen, J.L. (1994) Mature cystic teratoma: a clinicopathologic evaluation of 517 cases and review of the literature. *Obstet. Gynecol.*, **84**, 22-28.

Dawley, B., Acuna, A. & Grasu, B. (2012) Ectopic production of HCG by a benign ovarian mature cystic teratoma simulating an extra-uterine pregnancy: a case report. *W. V. Med. J.*, **108**, 15-17.

Diamanti-Kandarakis, E. & Papavassiliou, A.G. (2006) Molecular mechanisms of insulin resistance in polycystic ovary syndrome. *Trends Mol. Med.*, **12**, 324-332.

Freedman, D.D. & Scully, R.E. (1970) Case records of the Massachusetts General Hospital. Weekly clinicopathological exercises. Case 13-1970. *N. Engl. J. Med.*, **282**, 676-681.

Fritsch, M., Orfanos, C.E. & Zouboulis, C.C. (2001) Sebocytes are the key regulators of androgen homeostasis in human skin. *J. Invest. Dermatol.*, **116**, 793-800.

Hoffman, J.G., Strickland, J.L. & Yin, J. (2009) Virilizing ovarian dermoid cyst with leydig cells. *J. Pediatr. Adolesc. Gynecol.*, **22**, e39-40.

Ji, Q., Aoyama, C., Chen, P.K., Stolz, A. & Liu, P. (2005) Localization and altered expression of AKR1C family members in human ovarian tissues. *Mol. Cell. Probes*, **19**, 261-266.

Lopez-Beltran, A., Calanas, A.S., Jimena, P., Escudero, A.L., Campello, T.R., Munoz-Torres, M., Escobar-Jimenez, F., Carvia, R.E. & Nogales, F.F. (1997) Virilizing mature ovarian cystic teratomas. *Virchows Arch.*, **431**, 149-151.

Nakamura, Y., Hornsby, P.J., Casson, P., Morimoto, R., Satoh, F., Xing, Y., Kennedy, M.R., Sasano, H. & Rainey, W.E. (2009) Type 5 17beta-hydroxysteroid dehydrogenase (AKR1C3) contributes to testosterone production in the adrenal reticularis. *J. Clin. Endocrinol. Metab.*, **94**, 2192-2198.

Ozkaya, M., Sayiner, Z.A., Kiran, G., Gul, K., Erkutlu, I. & Elboga, U. (2015) Ectopic acromegaly due to a growth hormone-secreting neuroendocrine-differentiated tumor developed from ovarian mature cystic teratoma. *Wien. Klin. Wochenschr.*, **127**, 491-493.

Rotenberg, O., Shahabi, S. & Dar, P. (2009) Testosterone-secreting mature ovarian teratoma causing severe virilization in an adolescent: sonographic and color Doppler characteristics. *J. Ultrasound Med.*, **28**, 85-88.

Rutgers, J.L. & Scully, R.E. (1986) Functioning ovarian tumors with peripheral steroid cell proliferation: a report of twenty-four cases. *Int. J. Gynecol. Pathol.*, **5**, 319-337.

Sasano, H., Miyazaki, S., Sawai, T., Sasano, N., Nagura, H., Funahashi, H., Aiba, M. & Demura, H. (1992) Primary pigmented nodular adrenocortical disease (PPNAD): immunohistochemical and in situ hybridization analysis of steroidogenic enzymes in eight cases. *Mod. Pathol.*, **5**, 23-29.

Thiboutot, D., Martin, P., Volikos, L. & Gilliland, K. (1998) Oxidative activity of the type 2 isozyme of 17beta-hydroxysteroid dehydrogenase (17beta-HSD) predominates in human sebaceous glands. *J. Invest. Dermatol.*, **111**, 390-395.

Tomlinson, M.W., Alaverdian, A.A. & Alaverdian, V. (1996) Testosterone-producing benign cystic teratoma with virilism. A case report. *J. Reprod. Med.*, **41**, 924-926.

Tsujimoto, T., Takaichi, M., Endo, H., Yasuda, K., Kishimoto, M., Noto, H., Gomibuchi, H., Yasuda, H., Yamamoto-Honda, R., Takahashi, Y., Kajio, H., Sasano, H. & Noda, M. (2011) A patient with diabetes and breast cancer in whom virilization was caused by a testosterone-producing mature cystic teratoma containing a Brenner tumor. *Am. J. Med. Sci.*, **341**, 74-77.

Wu, D.H., McMurtrie, D.G., Hirsch, S.D. & Johnston, C.M. (2008) Postmenopausal hyperandrogenism caused by a benign cystic teratoma: a case report. *J. Reprod. Med.*, **53**, 141-144.